Published in: Steroids *(2022), vol. 187, 109096*
DOI: 10.1016/j.steroids.2022.109096
Status: Postprint (Author's version)

25-OH VITAMIN D CONCENTRATIONS MEASURED BY LC-MS/MS ARE EQUIVALENT IN SERUM AND EDTA PLASMA

Lisa N. van der Vorm[a], Caroline Le Goff[b], Stéphanie Peeters[b], Konstantinos Makris[c,d], Etienne Cavalier[b], Annemieke C. Heijboer[e,*]

[a] *Department of Clinical Chemistry, Central Diagnostic Laboratory, Amsterdam University Medical Center, Amsterdam, the Netherlands*

[b] *Department of Clinical Chemistry, University of Liège (ULiege), CHU de Liège, Sart Tilman, Liège, Belgium*

[c] *Clinical Biochemistry Department, KAT General Hospital, Athens, Greece*

[d] *Laboratory for Research of the Musculoskeletal System "Th. Garofalidis", Medical School, University of Athens, Athens, Greece*

[e] *Department of Clinical Chemistry, Endocrine Laboratory, Amsterdam Gastroenterology endocrinology & Metabolism, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands*

Keywords:

Vitamin D, 25(OH)D, LC-MS/MS, Serum, Plasma

Abstract

In contrast to a recent study reporting an unexpected significant difference for total 25-hydroxyvitamin D (25(OH)D) between serum and EDTA plasma, we demonstrate that concentrations of total 25(OH)D, $25(OH)D_2$, $25(OH)D_3$ and $24,25(OH)_2D_3$ do not differ between matched serum and EDTA plasma samples, using two well-characterized LC-MS/MS methods.

** Corresponding author.*

E-mail address: a.heijboer@amsterdamumc.nl (A.C. Heijboer).

Published in: Steroids *(2022), vol. 187, 109096*
DOI: 10.1016/j.steroids.2022.109096
Status: Postprint (Author's version)

Serum is the recommended sample type for vitamin D analysis, and vitamin D cut-off levels are based on serum concentrations. However, plasma is often used interchangeably with serum for the quantification of vitamin D and its metabolites. Previous studies with small sample sizes (n ≤ 25) did not find significant differences between heparin plasma, EDTA plasma and serum for liquid chromatography tandem mass spectrometry (LC-MS/MS) measurements of 25-hydroxyvitamin D_3 ($25(OH)D_3$) and 25-hydroxyvitamin D_2 ($25(OH)D_2$) in healthy adults [1,2]. In contrast, Harvey et al. recently reported that, with their LC-MS/ MS method, concentrations of total 25-hydroxyvitamin D (25(OH)D) were significantly higher in EDTA plasma samples compared to matched serum samples obtained from 114 pregnant women [3]. This was an unexpected finding since the sample preparation procedure prior to analysis by LC-MS/MS is meant to ensure a similar matrix composition. Therefore, we performed a similar comparison of vitamin D (metabolite) concentrations between matched EDTA plasma and serum samples with two LC-MS/MS methods, in two different centers.

Matched serum and plasma samples were fully anonymized leftover material (not specifically selected on any criteria) collected in serum and EDTA tubes (BD Vacutainer, BD, Plymouth, UK) at the Endocrine Laboratory of the Amsterdam University Medical Centre (Amsterdam UMC) in Amsterdam, the Netherlands. Ethics Committee approval was not required under the code of conduct for secondary use of human biomaterial in the Netherlands. Two aliquots of plasma and serum were stored at − 20 °C until analysis at the Endocrine Laboratory of the Amsterdam UMC, and two aliquots were transported on dry ice to the Department of Clinical Chemistry, University of Liège, Liège, Belgium. ` Concentrations of $25(OH)D_2$ and $25(OH)D_3$ were quantified using the in-house LC-MS/MS method of the laboratory in Amsterdam [4], whereas the laboratory in Liege quantified 25(OH)D` $_2$, $25(OH)D_3$, *Epi*-25 $(OH)D_3$ (the sum of which constituted total 25(OH)D) and 24,25 $(OH)_2D_3$ using a LC-MS/MS method certified as traceable to the Centers of Diseases Control (CDC) reference method according to the Vitamin D Standardization and Certification Program (VDSCP) [5]. Values below the limit of quantitation (LoQ) (equal to 2.45 nmol/l for $25(OH)D_3$, 4.5 nmol/l for $25(OH)D_2$, 0.65 nmol/l for $24,25(OH)_2D_3$ and 3.25 nmol/L for *Epi*-25(OH)D3 (all for the Liege method) and 4 nmol/l for both 25 ` $(OH)D_2$ and $25(OH)D_3$ measured with the Amsterdam method) were excluded from data analysis. *Epi*-$25(OH)D_3$ was above the LoQ in less than 10 (n = 8) matched samples, and was therefore only used for calculation of total 25(OH)D in these samples, not for further statistical comparison. Agreement of vitamin D concentrations between the two sample types was assessed using Pearson correlation, concordance correlation coefficient (CCC) and Passing and Bablok regression analysis.

A total of 39 matched serum and plasma samples were collected for quantification of vitamin D metabolites using the two LC-MS/MS methods. Serum $25(OH)D_2$ ranged between <4 and 60 nmol/l and serum $25(OH)D_3$ between 5.6 and 175 nmol/l (average of the two methods), thus covering the clinically relevant concentration range. 25 $(OH)D_2$ and $25(OH)D_3$ concentrations measured using the Amsterdam and Liege methods correlated well (R^2 0.994 and 0.992, CCC 0.983 and 0.995, respectively) The two methods were also not significantly different in Passing and Bablok regression analyses, as apparent from the intercepts (95 % CI) of 0.14 (− 1.08 to 1.02) and − 0.13 (− 1.97 to 1.25) and slopes of 1.06 (0.95 to 1.18) and 0.99 (0.96 to 1.03) for $25(OH)D_2$ and $25(OH)D_3$, respectively.

There was a strong correlation between vitamin D metabolite concentrations in serum versus EDTA plasma samples, with a R^2 ranging between 0.970 and 0.998 and a CCC ranging between 0.984

Published in: Steroids *(2022), vol. 187, 109096*
DOI: 10.1016/j.steroids.2022.109096
Status: Postprint (Author's version)

and 0.995 (Table 1). Passing and Bablok regression analysis demonstrated good agreement between the serum and plasma concentrations of all vitamin D metabolites, for both LC-MS/MS methods (Fig. 1A, B). Intercepts of all regression lines contained 0 in the 95 % confidence interval, indicating no systematic bias between the two sample types. The 95 % confidence interval of the slopes contained 1.00 for all regression lines, indicating no proportional bias, except for 25(OH)D_3 measured by the Amsterdam UMC method (95 % CI of the slope: 1.03 to 1.07). Due to the extremely strong correlation (r = 0.999) between the serum and plasma measurements with this method, the confidence interval around the regression line is very narrow, which makes this very small and clinically negligible difference statistically significant (Fig. 1A).

In conclusion, we demonstrated that concentrations of total 25(OH) D, 25(OH)D_2, 25(OH)D_3 and 24,25$(OH)_2D_3$, as measured by two different LC-MS/MS methods [4,5], did not differ between matched serum and plasma samples. This is particularly relevant information for the ongoing worldwide standardization efforts for vitamin D analysis. Of note, the observed matrix-independency for vitamin D quantification only applies to LC-MS/MS methods, as immunoassays suffer from considerably more matrix interference as well as lower specificity and sensitivity. Our findings are in contrast with the previously reported significant differences in total 25(OH)D levels between EDTA plasma and serum in pregnant women [3]. Although our study population was different, a large part of the discrepancy between sample types in the study by Harvey et al. may be explained by an apparently inferior analytical method, as there is not only a bias shown but also a relatively poor correlation coefficient (ranging from 0.862 to 0.910) for matched serum and plasma samples. It is therefore recommended that prior to the interchangeable use of serum and plasma in clinical and research settings, a similar validation of the locally available LC-MS/MS method is performed to ensure equivalence of vitamin D results.

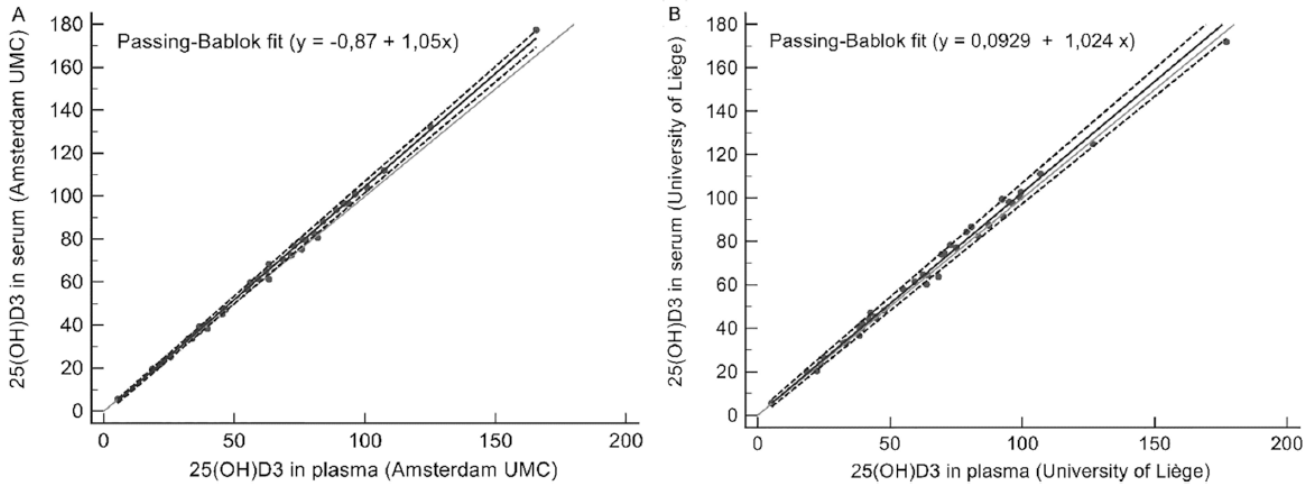
Table 1
Passing and Bablok regression analysis parameters for vitamin D metabolites concentrations in serum versus plasma samples.

	n	Mean (SD) in serum (nmol/l)	Mean (SD) in plasma (nmol/l)	R^2	CCC	Intercept (95 % CI)	Slope (95 % CI)
University of Liège							
Total 25(OH)D[a]	39	69.4 (30.7)	68.2 (30.8)	0.988	0.994	−0.17 (−2.37 to 2.73)	1.03 (0.98 to 1.07)
25(OH)D_2	11	16.4 (15.0)	16.8 (17.0)	0.992	0.987	0.10 (−0.95 to 1.83)	1.02 (0.86 to 1.16)
25(OH)D_3	39	64.8 (35.0)	63.5 (34.9)	0.992	0.996	0.09 (−1.34 to 1.68)	1.02 (0.99 to 1.05)
24,25$(OH)_2D_3$	35	1.4 (1.0)	1.4 (1.0)	0.970	0.984	0.04 (−0.04 to 0.11)	0.98 (0.91 to 1.05)
Amsterdam UMC							
25(OH)D_2	12	16.5 (17.0)	15.9 (15.8)	0.996	0.994	0.08 (−0.56 to 0.83)	1.03 (0.94 to 1.09)
25(OH)D_3	39	63.9 (35.5)	61.8 (33.4)	0.998	0.995	−0.87 (−1.76 to 0.00)	1.05 (1.03 to 1.07)

[a] sum of 25(OH)D_2, 25(OH)D_3 and *Epi*-25(OH)D_3. SD, standard deviation; R^2, Pearson correlation coefficient; CCC, concordance correlation coefficient.

Published in: Steroids *(2022), vol. 187, 109096*
DOI: 10.1016/j.steroids.2022.109096
Status: Postprint (Author's version)

Fig. 1.
Passing and Bablok regression analysis of 25(OH)D_3 concentration measured in serum versus plasma, for the Amsterdam (A) and Liège (B) method. The diagonal solid light grey line is the identity line; the solid black grey line is the best fit, with the 95% confidence interval indicated by the dashed black lines.



Author contribution statement

LV interpreted the results and drafted the manuscript. CG, SP acquired the data and revised the manuscript. AH, EC and KM conceived and designed the study, and revised the manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Data availability statement.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval.

Not applicable. Samples were leftover material not specifically selected on any criteria and were directly fully anonymized. Therefore, Ethics Committee approval was not required under the 'Code of conduct for secondary use of human biomaterial' in the Netherlands.

Published in: Steroids *(2022), vol. 187, 109096*
DOI: 10.1016/j.steroids.2022.109096
Status: Postprint (Author's version)

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] *N.S. Abu Kassim, F.P. Gomes, P.N. Shaw, A.K. Hewavitharana, Simultaneous quantitative analysis of nine vitamin D compounds in human blood using LC-MS/ MS, Bioanalysis. 8 (5) (2016) 397–411.*

[2] *A. Mena-Bravo, F. Priego-Capote, M.D. Luque de Castro, Study of blood collection and sample preparation for analysis of vitamin D and its metabolites by liquid chromatography-tandem mass spectrometry, Anal. Chim. Acta. 879 (2015) 69–76. [3] S.M. Harvey, V.E. Murphy, P.G. Gibson, M. Clarke, M.E. Jensen, The Impact of Sample Type on Vitamin D Quantification and Clinical Classification during Pregnancy, Nutrients. 12 (12) (2020) 3872.*

[4] *E.H.A.M. Elsenberg, E. ten Boekel, H. Huijgen, A.C. Heijboer, Standardization of automated 25-hydroxyvitamin D assays: How successful is it? Clin. Biochem. 50 (18) (2017) 1126–1130.*

[5] *N. Fabregat-Cabello, J. Farre-Segura, L. Huyghebaert, S. Peeters, C. Le Goff, J.- C. Souberbielle, E. Cavalier, A fast and simple method for simultaneous´ measurements of 25(OH)D, 24,25(OH)2D and the Vitamin D Metabolite Ratio (VMR) in serum samples by LC-MS/MS, Clin. Chim. Acta. 473 (2017) 116–123.*